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(54) Title: DNA ENCODING IgE RECEPTOR α-SUBUNIT OR FRAGMENT THEREOF

(57) Abstract

A cDNA sequence encoding the α-subunit of human mast cell IgE surface receptor or an IgE binding fragment thereof.

DNA ENCODING IgE RECEPTOR α-SUBUNIT OR FRAGMENT THEREOF

Background of the Invention

Mast cells, which are located in the connective tissue of higher vertebrates, store histamine, prostaglandins (local chemical mediators), and proteases within cytoplasmic granules. When stimulated (e.g., by immunological reaction), the contents of these granules are released from the mast cell. Histamine acts only on cells in its immediate vicinity and, upon release, causes blood vessels to dialate, thereby increasing their permiability to serum proteins (e.g., antibodies) and other immune system components (e.g., leukocytes). Histamine is largely responsible for the clincal symptoms of of "allergic reactions" such as hay fever (Metzger et al., 1986, Ann. Rev. Immunol. 4:419).

Immunological stimulation is mediated by IgE molecules, IgE being one of the five classes of antibodies found in higher vertebrates. IgE molecules bind with high affinity to an abundant, specific mast cell surface receptor. Bound IgE molecules, in turn, bind specific allergen molecules and considerable evidence indicates that the trigger for the release of the mast cell cytoplasmic granule contents is the allergen mediated cross-linking of two or more bound IgE molecules (Metzger et al., 1986; Ann. Rev. Immunol. 4:419; Ishizaka et al., 1977, J. Immunol. 119: 1589; Isersky et al., 1978, J. Immunol. 121:549; Froese, 1984, Prog. Allergy 34:142; Lewis and Austen, 1981, Nature 293:103).

The mast cell surface receptor consists of three subunits, a heavily glycosylated α-subunit of 50-60 kd exposed to the outer surface of the cell and bearing the IgE-binding site, and two non-glycosylated intramembrane components, the β and γ subunits, of approximately 30 and 20 kd, respectively (Metzer et al, 1986, Ann. Rev. Immunol. 4:419; Froese, 1984, Prog. Allergy 34:142).

Summary of the Invention

In general, the invention features a cDNA sequence encoding the α-subunit of human mast cell IgE surface receptor.

The invention also features a vector (plasmid or viral) containing DNA encoding the α-subunit of human mast cell IgE surface receptor.

The invention additionally features a soluble fragment of the α-subunit of human mast cell IgE surface receptor, such fragment being capable of binding to human IgE.

The human IgE receptor α-subunit, or fragments thereof, made according to the invention can be used in a variety of diagnostic and therapeutic applications, as will be explained in more detail below.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments, and from the claims.

Description of the Preferred Embodiments

The drawings will first be described.

Drawings

Fig. 1 is the HPLC elution profile of rat IgE surface receptor α-subunit tryptic digest fragments.

Fig. 2 is a a pair of restriction maps of two rat IgE receptor α-subunit clones.

Fig. 3 is a restriction map of a human IgE receptor α-subunit clone.

Fig. 4 is a diagrammatic comparison of the DNA sequences of rat and human IgE receptor α-subunit cDNA clones.

The cDNA sequence encoding human mast cell IgE surface receptor α-subunit was produced according to the following general series of steps. First, rat IgE surface receptor α-subunit was purified, fragmented, and tryptic peptides produced. A rat cDNA clone was then isolated using oligonucleotides designed on the basis of the amino acid sequence of one of the tryptic peptides. A human cDNA library was then prepared, and the rat cDNA fragments were used to screen the human library. In more detail, these procedures were carried out as follows.

Rat IgE receptor protein purification, tryptic peptide <u>preparation, and sequence determination</u>

Rat basophilic leukemia (RBL-2H3) cells were solubilized and incubated overnight at 4°C with monoclonal anti-rat mast cell IgE receptor antibody (mAb BC4) coupled to Sepharose 4B beads (Basciano et al., 1986, J.B.C., Vol. 261, page 11823). The beads were washed and the bound proteins were eluted with 5% acetic acid and then lyophilized. Aliquots were analyzed by $NaDodSO_4$-PAGE (Laemmli, 1970, Nature <u>227</u>:680) followed by silver staining (Oakley et al., 1980, Anal. Biochem. <u>105</u>:361). As expected, there were bands corresponding to α, ß, and γ chains of the receptor. The different receptor components were further purified by elution from $NaDodSO_4$-PAGE.

Amino acid sequence determination by N-terminal analysis was inappropriate because the N-terminal end of the eluted α-subunit samples appeared to be blocked. The samples therefore were reduced with 2 mM

dithiothreitol in 6M guanidine HCl, 100 mM Tris (pH 8.3) and 1.0 mM EDTA at 37°C under N_2 and were then S-carboxymethylated with 10 mM iodoacetic acid. Salts were removed by HPLC on a Vydac C_4 column. Desalted samples were treated with TPCK (L-1-tosylamido-2-phenylethyl chloromethyl ketone) treated trypsin in 100 mM Tris (pH 7.2). The resulting tryptic peptides were separated by HPLC on a Vydac C_4 column; the elution profile is shown in Fig. 1. Peaks indicated by arrows were subjected to amino acid sequencing using an Applied Biosystems vapor phase amino acid sequencer. Peptide sequences obtained from these peaks are shown in Table 1, below.

Table 1

WIHNDSISNXK and	(Peak 1)
YSYDSNXISIR	
ILTGDKVTLIXNG	(Peak 2)
VIYYK	(Peak 3)
SVVSLDPPWIR	(Peak 4)

Isolation of rat mast cell IgE receptor α-subunit cDNA clones and nucleotide sequence determination

The strategy for isolating rat IgE receptor α-subunit cDNA was to synthesize oligonucleotides predicted to be complementary to the rat α-subunit gene, and then use those oligonucleotides to screen a rat cDNA library.

Oligonucleotide Probes

Computer assisted analysis of the tryptic fragments was carried out using software versions 4 and 5 from the Genetics Computer Group of the University of Wisconsin (Devereux et al., Nucl. Acids Res. 12:7035). Among the peptide sequences, peptide 4 (Fig. 1, peak 4) showed significant homology to a sequence near the

NH_2-terminus of the mouse Fcγ (IgG) receptor (Ravetch et al., 1986, Science 234:718), suggesting an analogous sequence in the IgE receptor subunit. From the sequence of peptide 4, the least codon-redundant portion, Asp-Pro-Pro-Trp-Ile, was chosen to make a 32-mixture of the 14mer oligonucleotide, 5'-ATCCA(A/G/C/T)GG(A/G/C/T) GG(A/G)TC-3' using an automated DNA synthesizer (Models 380A and B, Applied Biosystems). The oligonucleotides were labeled using ^{32}P as described in Maniatis et al. (1982), Molecular Cloning.

Construction and Screening of cDNA Libraries

RNAs were extracted from rat RBL-2H3 cells by homogenizing in 6M guanidinium isothiocyanate followed by ultracentrifugation over a CsCl cushion as described in Maniatis et al. (1982) Molecular Cloning, followed by phenol-chloroform-isoamyl alcohol (25:24:1) extraction. Poly(A)+ RNAs were prepared using oligo(dT) cellullose columns (Aviv and Leder, 1972, Proc. Natl. Acad. Sci. U.S.A. 69:1408), and cDNA libraries were constructed as described in Okayama and Berg, 1983, Molec. Cell. Biol. 3:280 using slightly modified vector and linker fragments (Noma et al., 1986, Nature 319:640). From 5 μg of poly (A)+ RNA, 9×10^5 independent colonies were obtained.

About 7×10^4 independent colonies were screened with labelled oligonucleotide probe, by the method described in Hahahan and Meselson, 1980, Gene 10:63, and three positive clones were identified. The nucleotide sequences of two of the three clones which showed similar restriction enzyme digestion patterns were determined by the dideoxy chain termination method described in Sanger et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463 using alkali-denatured plasmid DNA.

Referring to Figs. 2 and 4, respectively, these clones have exactly the same sequence except for one deletion (bp 21) in pAS-r-IgER-5A (cl 5A) and two deletions (bp 163 and 8) in pAS-r-IgER-2A (cl 2A). A full length cDNA clone (cl 2A/5A) was constructed by combining the left one-third of the cl 2A cDNA with the right two-thirds of the cl 5A cDNA. In RNase protection experiments (Melton et al., 1984, Nucl. Acids Res. 12:7035) using a labelled RNA transcribed from the clone 2A/5A cDNA in pGEM3, a plasmid containing the promoter sequence for T7 RNA polymerase, the majority of the IgE receptor mRNA was shown to have no deletions. This observation permitted the primary structure of rat IgE receptor α-subunit to be deduced from the 735 bp open reading frame of the undeleted sequence (Fig. 4). That open reading frame was found to encode a 245 amino acid peptide containing perfect matches to the peptide sequences which had been determined by amino acid sequencing.

Isolation of the human mast cell IgE receptor α-subunit cDNA clone

To clone the human α-chain cDNA, a cDNA library was prepared, generally as outlined above, from a human mast cell line known to produce IgE receptors (KU812). About 9×10^4 colonies were screened with nick-translated HpaII(46)-PvuII(970) fragment of rat cDNA clone 2A/5A. Hybridization was carried out in 6X SSC -50% formamide -10% dextran sulfate at 42°C, 15 hours, and the filters were then washed twice in 0.1X SSC and 0.1% $NaDodSO_4$ at 55°C for 15 minutes. Three positive colonies were identified, and the one which had the largest insert (pAS-h-IgER-110B) was further characterized. Both nucleotide and deduced amino acid sequences were compared with the rat sequence (Fig. 4).

Insertion into an Expression Vector

The human cDNA sequence of the invention can be inserted, by conventional techniques, into any of a variety of expression vectors to produce recombinant human mast cell IgE receptor α-subunit of the invention. Shortened sequences can be used for the production of soluble fragments.

The cDNA encoding the human mast cell IgE surface receptor α-subunit can, for example, be inserted into the expression vector described in Ringold U.S. Pat. No. 4,656,134, hereby incorporated by reference. This plasmid can then be used to transform mammalian host cells, and the human mast cell IgE receptor α-subunit can be isolated and purified according to conventional methods.

In its unglycosylated form, the polypeptide can be produced in a bacterial host, e.g., E. coli. The cDNA encoding the human mast cell IgE surface receptor α-subunit can, for example, be inserted into the expression vector described in DeBoer et al., 1983, Proc. Natl. Acad. Sci. 80:21. The plasmid, which carries the hybrid tac promoter, can be used to transform E. coli, and the α-subunit can be isolated and purified according to conventional methods.

Use

The human mast cell IgE surface receptor α-subunit of the invention, or a fragment thereof, can be used to produce anti-IgE surface receptor polyclonal or monoclonal antibodies using conventional methods. These antibodies can be used in an in vitro diagnostic assay, of any standard format, e.g., ELISA, to determine the level of IgE receptor in a biological sample obtained from a human patient, e.g., blood or tissue samples, in particular, in basophils. The amount

of IgE receptor α-subunit present in the sample can serve as a measure of the allergic response of the patient to a substance to which the patient has been exposed. IgE receptor α-subunit levels can also be measured to determine the efficiency of anti-allergy therapies, and to monitor a patient's allergic status over time. The antibodies can also be used in the immunochromatographic purification of IgE receptor α-subunit from culture media.

The IgE receptor α-subunit, or soluble fragments thereof, can also be used therapeutically to treat human patients suffering from allergies. The IgE receptor α-subunit or fragment thereof competes for IgE with the receptor naturally present on mast cells, so that IgE is bound to the administered peptide and unable to bind to mast cells to mediate the allergic response. As an alternative to using the peptide itself in competitive inhibition therapy, the peptide can be used to design non-peptide drugs which behave therapeutically like the peptides. Generally, X-ray crystallography is used to elucidate the three-dimensional structure of the peptide, in particular its IgE binding sites, and a non-peptide compound is synthesized, with the aid of computer modelling, to mimic the functionally important regions of the peptide.

The peptide, or compound synthesized on the basis of the structure of the peptide, will be administered as an unmodified peptide or in the form of a pharmaceutically acceptable salt, admixed with a physiologically acceptable carrier, e.g., saline. Examples of preferred salts are those of therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pamoic acid, as

well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids such as hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid. The composition can be in the form of a liquid, for intravenous, subcutaneous, parenteral, or intraperitoneal administration, or a spray for bronchial or nasal administration.

The IgE receptor α-subunit can also be used to screen substances which potentially have the capacity to bind to the IgE receptor α-subunit; such substances, when administered therapeutically to a human patient suffering from an allergy, can alleviate the allergic response by binding to the IgE receptor α-subunit on the patient's mast cells, thus preventing IgE from binding and thereby interrupting the IgE-mediated allergic response. Screening for such IgE receptor α-subunit binding substances can be carried out by immobilizing the α-subunit, bringing the substance to be screened, in labeled form (e.g., radiolabeled), into contact with the immobilized subunit, separating soluble from immobilized phases, and detecting bound label as an indication of binding.

Deposit

The cDNA encoding the IgE receptor α-subunit was deposited in the American Type Culture Collection, Rockville, Maryland, on December 1, 1987 and given ATCC Accession No.______________. One of applicants' assignees, President and Fellows of Harvard College, hereby acknowledge their responsibility to replace this culture should it die before the end of the term of a patent issued hereon, 5 years after the last request for a culture, or 30 years, whichever is the longer, and their responsibility to notify the depository of the issuance of such a patent, at which time the deposit

will be made irrevocably available to the public. Until that time the deposit will be made available to the Commissioner of Patents under the terms of 37 CFR Section 1-14 and 35 USC Section 112.

Other Embodiments

Other embodiments are within the following claims. For example, the soluble α-subunit fragment of the invention need not have perfect homology with the corresponding region of the naturally-occurring molecule, but need only have sufficient homology (generally, at least 75%) to bind to human IgE. The fragment also must be large enough (generally, at least ten amino acids) to bind IgE. If solubility is desired, the fragment preferably should contain none of the hydrophobic transmembrane portion of the naturally occurring molecule. Fragments of the α-subunit, as well as the whole molecule, can be used to raise antibodies, useful as described above for diagnostic and purification purposes.

Claims

1. A cDNA sequence encoding the α-subunit of human mast cell IgE surface receptor or an IgE binding fragment thereof.

2. A vector comprising DNA encoding the α-subunit of the human mast cell IgE surface receptor or an IgE binding fragment thereof.

3. A soluble fragment of the α-subunit of human mast cell IgE surface receptor, said fragment being capable of binding to human IgE.

4. Recombinant α-subunit of human mast cell IgE surface receptor.

FIG. 1

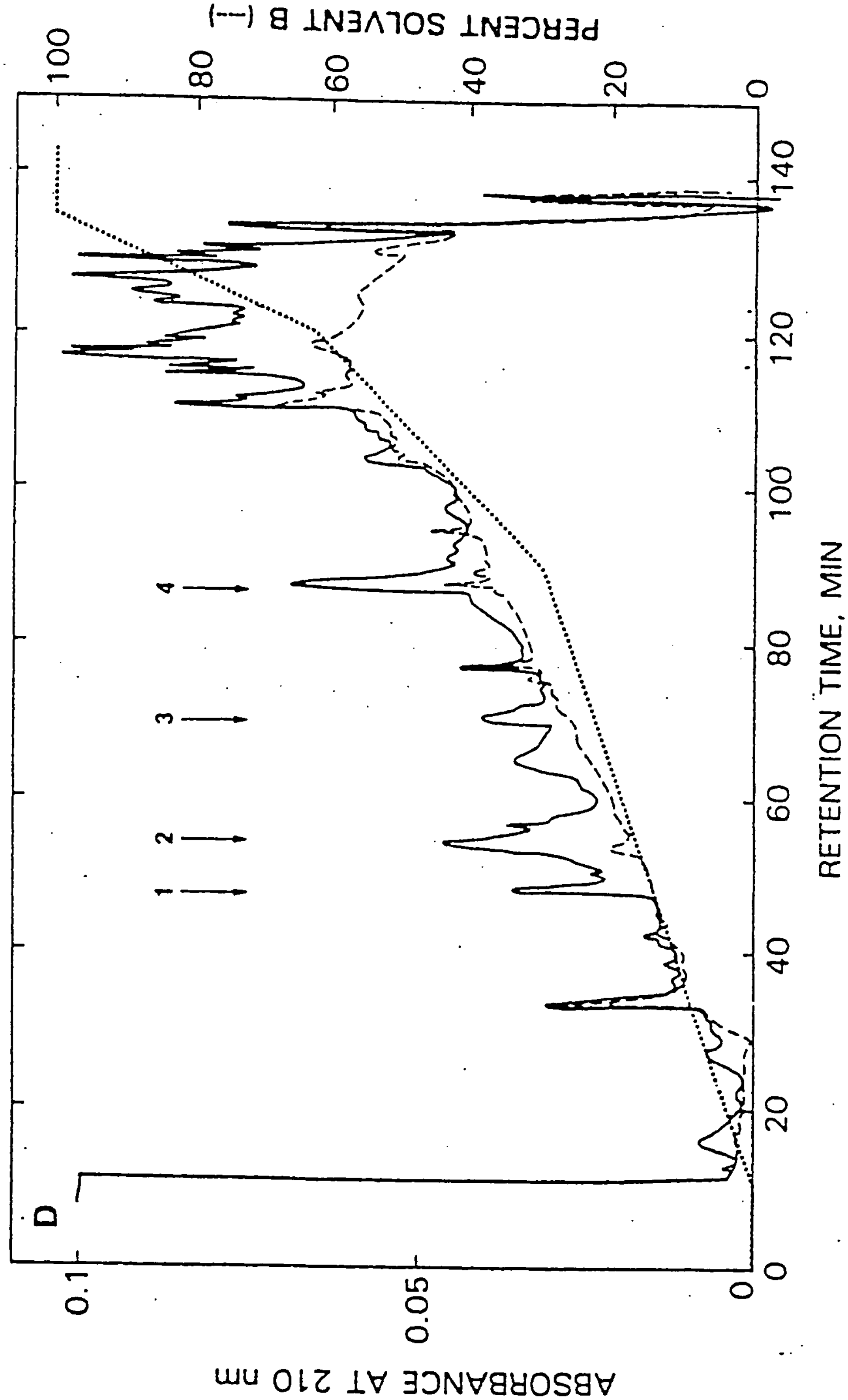
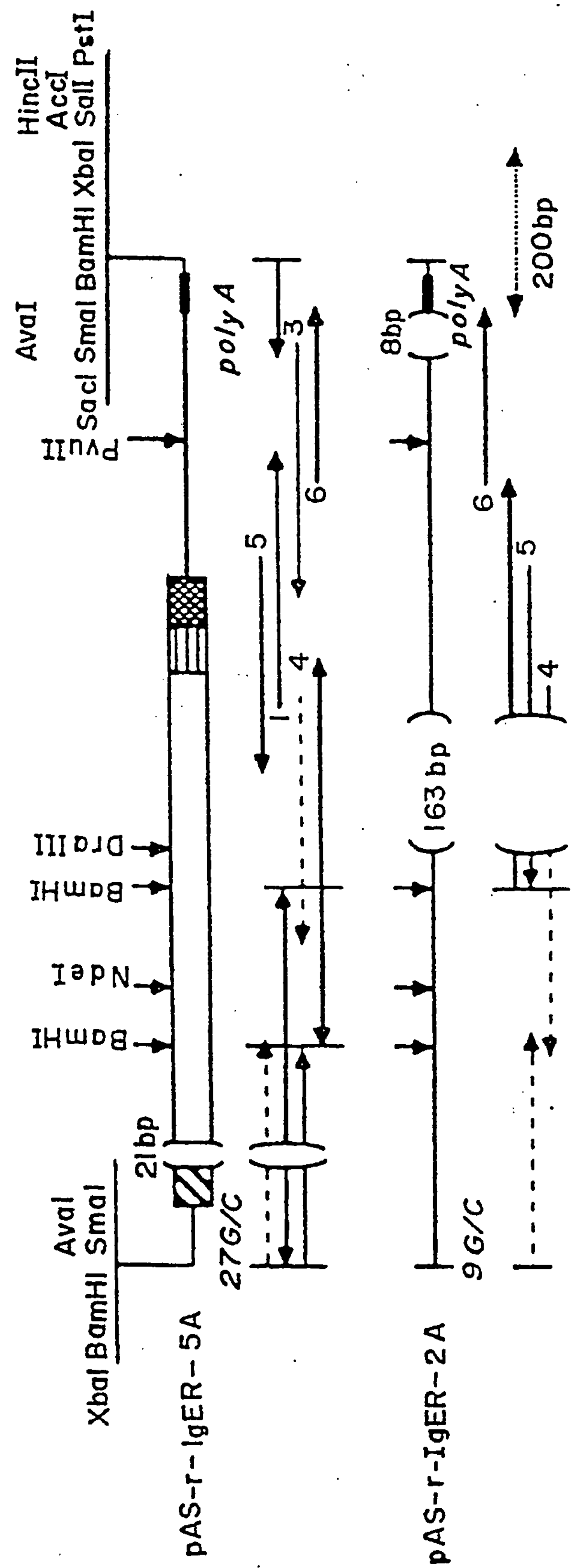

PERCENT SOLVENT B (---)
100
80
60
40
20
0
D
1
2
3
4
0.1
0.05
0
ABSORBANCE AT 210 nm
0
20
40
60
80
100
120
140
RETENTION TIME, MIN

FIG. 2


HincII
AccI
XbaI BamHI XbaI SalI PstI
AvaI
SacI SmaI BamHI
PvuII
poly A
3
6
5
1
4
DraIII
BamHI
NdeI
BamHI
21bp
AvaI
SmaI
XbaI BamHI
27G/C
pAS-r-IgER-5A
8bp
poly A
163 bp
9G/C
pAS-r-IgER-2A
200bp

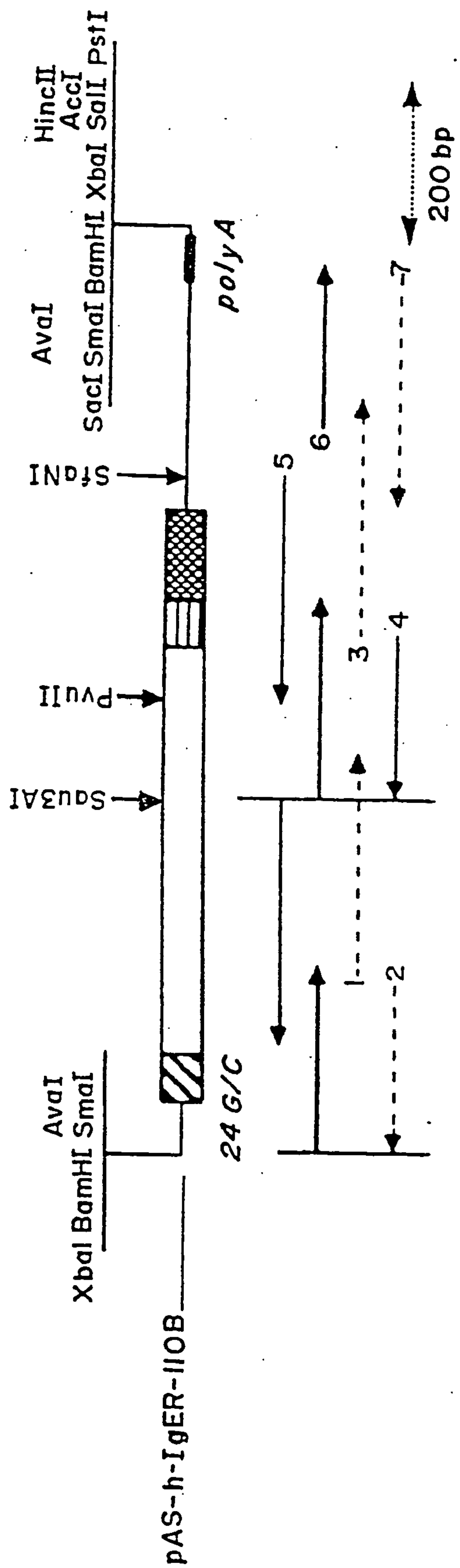

HincII
AccI
SacI SmaI BamHI XbaI SalI PstI
AvaI
poly A
SfaNI
PvuII
Sau3AI
AvaI
XbaI BamHI SmaI
24 G/C
pAS-h-IgER-110B
1
2
3
4
5
6
7
200 bp

FIG. 3

FIG. 4a

```
Rat     ACTTTTGAAGCCGTAGCTCACTGGTGCAGTTAGCACCTGAAGGCACAGGG
                                 ||  |  |||| |||||| | || |   |
Human   ..................AC-AGCACAGTAAGCACCAGGAGTCCATGAA

           1                                     10
           M  D  T           G  G  S  A  R  L  C  L  A  L  V
Rat     GCA-ATGGATACT-------GGAGGATCTGCCCGGCTGTGCCTAGCATTAGT
        | | |||| | ||        | |      || | |  |||||  |||| ||| |
Human   GAAGATGGCTCCTGCCATGGAATCCCCTACTCTACTGTGTGTAGCCTTACT
          (M) A  P  A  M  E  S  P  T  L (L)(C) V (A)(L) L

                             20                               30
           L  I  S  L  G  V  M  L  T  A  T  Q  K  S  V  V  S
Rat     GCTCATATCTCTGGGTGTCATGCTAACAGCCACTCAGAAATCTGTAGTGTC
        | || |   |||  | || | || || ||| | |||||||| ||   || ||
Human   GTTCTTCGCTCCAGATGGCGTGTTAGCAGTCCCTCAGAAACCTAAGGTCTC
           F  F  A  P  D  G  V (L) A  V  P (Q)(K) P  K (V)(S)

                                 40
           L  D  P  P  W  I  R  I  L  T  G  D  K  V  T  L  I
Rat     CTTGGACCCACCGTGGATTAGAATACTTACAGGAGATAAAGTGACTCTTAT
        |||| |||| || |||| ||||||| ||| |||||| || ||||||||||
Human   CTTGAACCCTCCATGGAATAGAATATTTAAAGGAGAGAATGTGACTCTTAC
          (L) N (P)(P)(W) N (R)(I) F  K (G) E  N (V)(T)(L) T

           50                                   60
           C  N  G  N  N  S  S  Q  M  N  S  T  K  W  I  H  N
Rat     ATGCAATGGGAACAATTCCTCTCAAATGAACTCTACTAAATGGATCCACAA
        ||| |||||||||||||| || |  || |  |  .|| || |||||| |||||||
Human   ATGTAATGGGAACAATTTCTTTGAAGTCAGTTCCACCAAATGGTTCCACAA
          (C)(N)(G)(N)(N) F  F  E  V  S (S)(T)(K)(W) F (H)(N)

                           70                             80
           D  S  I  S  N  V  K  S  S  H  W  V  I  V  S  A  T
Rat     TGATAGCATCTCTAATGTGAAATCGTCACATTGGGTCATTGTGAGTGCCAC
        || ||| | || | | || |     |||  || |    ||||||| |||||
Human   TGGCAGCCTTTCAGAAGAGACAAATTCAAGTTTGAATATTGTGAATGCCAA
           G (S) L (S) E  E  T  N (S) S  L  N (I)(V) N (A) K

                                 90
           I  Q  D  S  G  K  Y  I  C  Q  K  Q  G  F  Y  K  S
Rat     CATTCAAGACAGTGGAAAATACATATGTCAGAAGCAAGGATTTTATAAGAG
          || ||||||||||| |||||| ||||||| | |||    | || || ||||
Human   ATTTGAAGACAGTGGAGAATACAAATGTCAGCACCAACAAGTTAATGAGAG
           F  E (D)(S)(G) E (Y) K (C)(Q) H (Q) Q  V  N  E (S)
```

FIG. 4b

```
       100                       110
       K  P  V  Y  L  N  V  M  Q  E  W  L  L  L  Q  S  S
Rat    CAAACCTGTGTACTTGAACGTGATGCAAGAGTGGCTGCTGCTCCAATCTTC
Human  TGAACCTGTGTACCTGGAAGTCTTCAGTGACTGGCTGCTCCTTCAGGCCTC
       E (P)(V)(Y)(L) E (V) F  S  D (W)(L)(L)(L)(Q) A (S)

             120                             130
       A  D  V  V  L  D  N  G  S  F  D  I  R  C  R  S  W
Rat    TGCTGACGTGGTCTTAGACAATGGATCCTTTGACATCAGATGCCGTAGCTG
Human  TGCTGAGGTGGTGATGGAGGGCCAGCCCCTCTTCCTCAGGTGCCATGGTTG
      (A) E (V)(V) M  E  G  Q  P  L  F  L (R)(C) H  G (W)

                         140                      150
       K  K  W  K  V  H  K  V  I  Y  Y  K  D  D  I  A  F
Rat    GAAGAAATGGAAAGTCCACAAGGTGATCTACTACAAGGACGACATTGCTTT
Human  GAGGAACTGGGATGTGTACAAGGTGATCTATTATAAGGATGGTGAAGCTCT
       R  N (W) D (V) Y (K)(V)(I)(Y)(Y)(K)(D) G  E (A) L

                                  160
       K  Y  S  Y  D  S  N  N  I  S  I  R  K  A  T  F  N
Rat    CAAGTACTCTTATGACAGCAACAACATCTCCATTAGAAAGGCCACATTTAA
Human  CAAGTACTGGTATGAGAACCACAACATCTCCATTACAAATGCCACAGTTGA
      (K)(Y) W (Y) E  N  H (N)(I)(S)(I) T  N (A)(T) V  E

          170                            180
       D  S  G  S  Y  H  C  T  G  Y  L  N  K  V  E  C  K
Rat    TGACAGTGGCAGCTACCACTGCACAGGCTATTTGAACAAGGTTGAATGTAA
Human  AGACAGTGGAACCTACTACTGTACGGGCAAAGTGTGGCAGCTGGACTATGA
      (D)(S)(G) T (Y) Y (C)(T)(G) K  V  W  Q  L  D  Y  E

                      190                       200
                                      I  T  Q  L  S  I  V
       S  D  K  F  S  I  A  V  V  K  D  Y  T  I  E  Y  R
Rat    ATCTGATAAATTCAGTATTGCTGTAGTAAAAGATTACACAATTGAGTATCG
Human  GTCTGAGCCCCTCAACATTACTGTAATAAAAGCT---CCGCGTGAGAAGTA
      (S) E  P  L  N (I) T (V) I (K) A  -  P  R (E) K  Y
```

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FIG. 4c

```
                                  210
        G  Y  N  S  F  S  H  H  W  R  *
        W  L  Q [L  I  F  P  S  L  A  V  I  L  F  A  V  D]
Rat     TTGGCTACAACTCATTTTCCCATCATTGGCGGTGATTCTGTTTGCTGTGGA
        ||||||||| |  || ||||||  |||| ||||||||||||||||||||||
Human   CTGGCTACAATTTTTTATCCCATTGTTGGTGGTGATTCTGTTTGCTGTGGA
        (W)(L)(Q) [F  F  I (P) L (L) V (V)(I)(L)(F)(A)(V)(D)]

          220                         230
        [T  G  L  W  F] S  T  H  K  Q  F  E  S  I  L  K  I
Rat     CACTGGGTTATGGTTCTCAACCCACAAACAGTTCGAATCCATCTTGAAGAT
        ||| || ||||   ||||||| ||   | ||| ||  ||   ||||||||||
Human   CACAGGATTATTTATCTCAACTCAGCAGCAGGTCACATTTCTCTTGAAGAT
        [(T)(G)(L) F  I] (S)(T) Q  Q (Q) V  T  F  L (L)(K)(I)

                     240
        Q  K  T  G  K  G  K  K  K  G  *
Rat     TCAGAAGACTGGAAAAGGCAAAAAAAAAGGTTGAAACCTAACTCTTAACCA
        | |||  ||  | ||||||     | |    |||| ||  |   || | |||
Human   TAAGAGAACCAGGAAAGGC--TTCAGACTTCTGAACCCACATCCTAAGCCA
        K  R (T) R (K)(G)    F  R  L  L  N  P  H  P  K  P

Rat     AGGTATATAAAGGAACTAATGTCATCGCTTAAGAGACAATTCTTAACAATT
        |       | |||  |||| || | ||  || |||| |  |||   ||| |||
Human   AACCCCA-AAAACAACTGATATAATTACTCAAGA-AATATTTGCAAC-ATT
        N  P  K     N  N  *

Rat     A--TTTCCCACAGTATCTTCAATAGCCTTTCAACTGTCAAAGGACA-CTCA
        |  |||     ||| |||   |||| ||    |||| |||||||   ||| ||
Human   AGTTTTTTTCCAGCATCAGCAATTGCTACTCAATTGTCAAA-CACAGCTTG

Rat     TGTTATCCATAGAAATGTCTGTACCCCAGGAATTGCATAAATGCTTCATTA
            ||| |||||||| |||||| | | ||||| ||   | |||||||||||||
Human   CAATATACATAGAAACGTCTGTGCTCAAGGATTTATAGAAATGCTTCATTA

Rat     AACCAACAGCAGCTGGTTAAGTGACATGCAATAAATAACAAATACTCAATA
        |||  |  | | ||||||||||| ||||        |||| || | ||||||
Human   AACTGAGTGAAACTGGTTAAGTGGCATG----TAATAGTAAGTGCTCAATT

Rat     AACACTGGTTTAAGAAGTTAAAGAATGAGCTGATTGCTTTGTCTATATCTG
        |||| ||||| || || | | |||||||     ||||  ||| |      || ||
Human   AACATTGGTTGAATAAATGAGAGAATGAATAGATTCATTTATTAGCATTTG
```

FIG. 4d

```
Rat     TAAAAGAGAAGTACAATTTGAATAAAGTATGGAATCATGT[AGAATGGT]AAA
        ||||||||| || |||||| |||||| ||     ||| ||   |     ||||
Human   TAAAAGAGATGTTCAATTTCAATAAAATA---AAT-ATAAAACCATGTAAA

Rat     AAAA(polyA)
        ||||
Human   AAAA(polyA)
```

INTERNATIONAL SEARCH REPORT

International Application No PCT/US88/04255

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4):C12N 15/00; C07H 21/04; C07K 13/00
U.S. CL.: 435/320; 536/27; 530/350,387,388

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
U.S.	435/320; 536/27; 530/350, 387, 388

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

Online Computer Search of Chemical Abstracts 1967-1988
Search Terms: IgE surface receptor proteins

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A,P	Biochemistry, Volume 26, issued 1987 (U.S.A.) KINET, "A cDNA Presumptively Coding for the α-subunit of the Receptor With High Affinity for Immunoglobulin E". See pages 4605-4610.	1-4
Y	J. Immunology, Volume 123, No. 5, issued November 1979 (U.S.A.) HEMPSTEAD, "Characterization of the IgE Receptor Isolated from Human Basophils". See pages 2283-2291.	3-4
Y	Federation Proceedings, Volume 41, No. 1, issued January 1982 (U.S.A.) METZER, "Structure of the High-Affinity Mast Cell Receptor for IgE". See pages 8-11.	3-4

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
31 January 1989	12 APR 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	BLONDEL HAZEL